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MicroMator: Open and Flexible Software for Reactive Microscopy

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13 Abstract

14 *Microscopy image analysis has recently made enormous progress both in terms of accuracy and speed*
15 *thanks to machine learning methods. This greatly facilitates the online adaptation of microscopy*
16 *experimental plans using real-time information of the observed systems and their environments. Here*
17 *we report MicroMator, an open and flexible software for defining and driving reactive microscopy*
18 *experiments, and present applications to single-cell control and single-cell recombination.*

19

20 Introduction

21 Software for microscopy automation are essential to support reproducible high-throughput
22 microscopy experiments¹. Samples can now be routinely imaged using complex spatial and temporal
23 patterns. Yet, in the overwhelming majority of cases, executions of experiments are still cast in stone
24 at the beginning, with little to no possibility for human or computer-driven interventions during the
25 experiments. This is all the more surprising given that image analysis has recently made a giant leap
26 in terms of accuracy and rapidity thanks to deep learning methods, thus opening the way for
27 implementing elaborate protocols. Software empowering microscopy with real-time adaptation
28 capabilities is needed to exploit the full potential of automated microscopes.

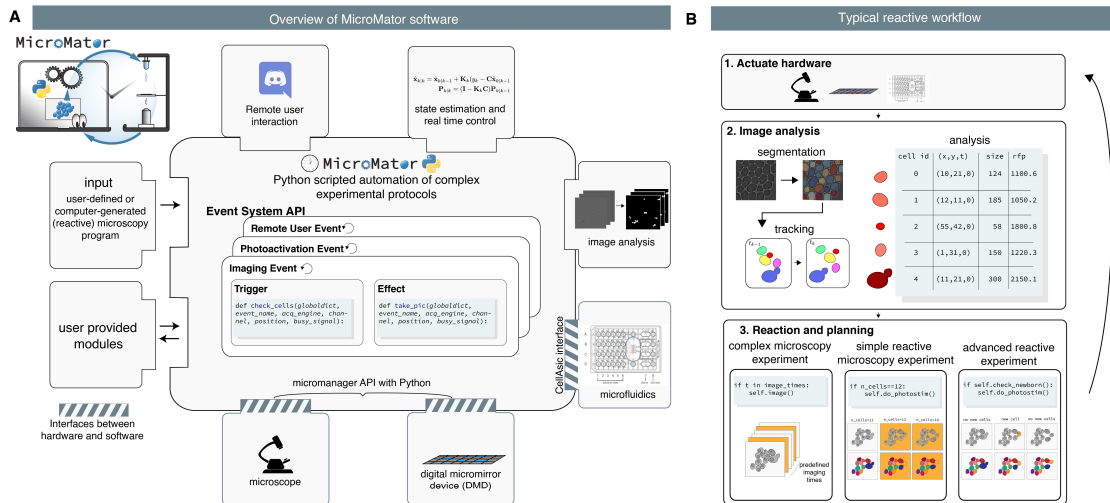
29 Several dedicated microscopy software solutions have been developed for applications requiring real-
30 time analysis. This is notably the case for the efficient scanning of large and complex microscopy
31 samples (eg, Refs²⁻⁶). For other important applications, such as real-time control of cellular processes
32 (eg, Refs⁷⁻¹³), results are generally obtained using ad hoc software solutions. Very few generic tools
33 have been developed so far to facilitate the realization of complex, reactive microscopy experiments.
34 One notable exception is Pycro-Manager¹⁴. This powerful framework is built on top of μ Manager, a
35 widely-used software^{15,16} controlling a large range of microscopy hardware. In Pycro-Manager,
36 reactive protocols are built from the ground up. Whereas this gives maximal flexibility, this also
37 increases the difficulty to rapidly design experiments, especially for non-expert users. Moreover, no
38 in-depth case studies demonstrating its practical applicability –and showing possible limitations– have
39 been reported so far. One can also mention Cheetah, a simple to use Python library to support the
40 development of real-time cybergenetic control platforms that combines microscopy imaging and
41 microfluidics control¹⁷. In its current state, the possibilities to programmatically control the
42 microscope appear limited.

43 **MicroMator Software**

44 In this paper, we present MicroMator, a simple software solution supporting reactive microscopy
45 experiments, and demonstrate its potential via two challenging case studies. In MicroMator, events
46 play a fundamental role (Fig 1A). They consist of Triggers and Effects. They can be defined by the user
47 in a flexible manner. Examples of triggers include "at the 10th frame", "if more than 100 cells are in
48 the field of view", "if the fluorescence of the 3rd newborn cell exceeds 100 a.u." and "if message
49 !update position=10 frame=last is received from Discord". Examples of effects include changing a
50 microscope configuration, sending light in the field of view with a given pattern, actuating a
51 microfluidic pump, or starting an optimization routine. Microscopy experiments are defined by a main
52 image acquisition loop, that serves as a backbone for the experiment, and by event creation functions
53 (Fig 1B). Naturally, the main acquisition loop itself can be modified by event effects in the course of
54 the experiment. MicroMator is written in Python 3, is open-source and has a modular design. For
55 controlling hardware, MicroMator primarily uses the powerful Python API of μ Manager pymmcore,
56 but can also use other dedicated Python or web-based APIs provided by vendors, as done for our
57 CellAsic ONIX microfluidic platform. Various types of analysis can be performed using dedicated
58 software modules, such as on-line image analysis or real-time control and optimization.
59 Communication modules can be used to interface MicroMator with digital distribution platforms such
60 as Discord to track experiment progress and potential issues. Lastly, MicroMator leverages Python's
61 multiprocessing module to perform computations concurrently and possesses an extensive and
62 customizable logging system, gathering logs of all modules in a unique file and fostering reproducible
63 research (see SI Text).

64 We also provide SegMator, a software which uses U-Net for bright-field yeast cell segmentation
65 (provided by DeLTA¹⁸), and tracking using TrackPy¹⁹. U-Net is a convolutional neural network with a
66 structure that excels at image segmentation²⁰. U-Net can analyze dense fields of cells in a few seconds
67 and with good accuracy (see SI Text and Fig S1 and S2 and Movie S1).

68 To showcase the full potential of reactive experiments performed with MicroMator, we designed
69 experiments in which cellular processes are controlled in real-time. Single-cell stimulations are
70 computed on line based on the cell state and/or position, demonstrating that reactive loops can be
71 implemented at the level of individual cells. These experiments are inspired by previously-published
72 studies^{10,11,13,21} and show how these could be repeated and further extended using generic software.
73 We also provide a tutorial application in which cells with fluorescent proteins are imaged with
74 increasing durations such that the measured intensity reaches a given threshold (Supplementary Text
75 1). This could typically be used to guarantee a good signal to noise ratio irrespectively of the initial
76 fluorescence of the cells.



77

78

Figure 1. MicroMator overview. **A.** Modular software architecture. MicroMator consists of an extensible set of modules that control various hardware and software aspects of microscopy experiments and of a central core that handles user-defined events. It is written in the high-level programming language Python. **B.** Event-based reactive microscopy workflow. Imaging can be followed by online analysis of the samples. This typically involves segmentation, tracking, quantification of cell properties, and possibly advanced additional computations. Effects may then be triggered based on the result of the analysis. These may include the physical actuation of the hardware or the initiation of communications or of additional computations.

84

85 Model predictive control of gene expression at the single-cell level in yeast

86

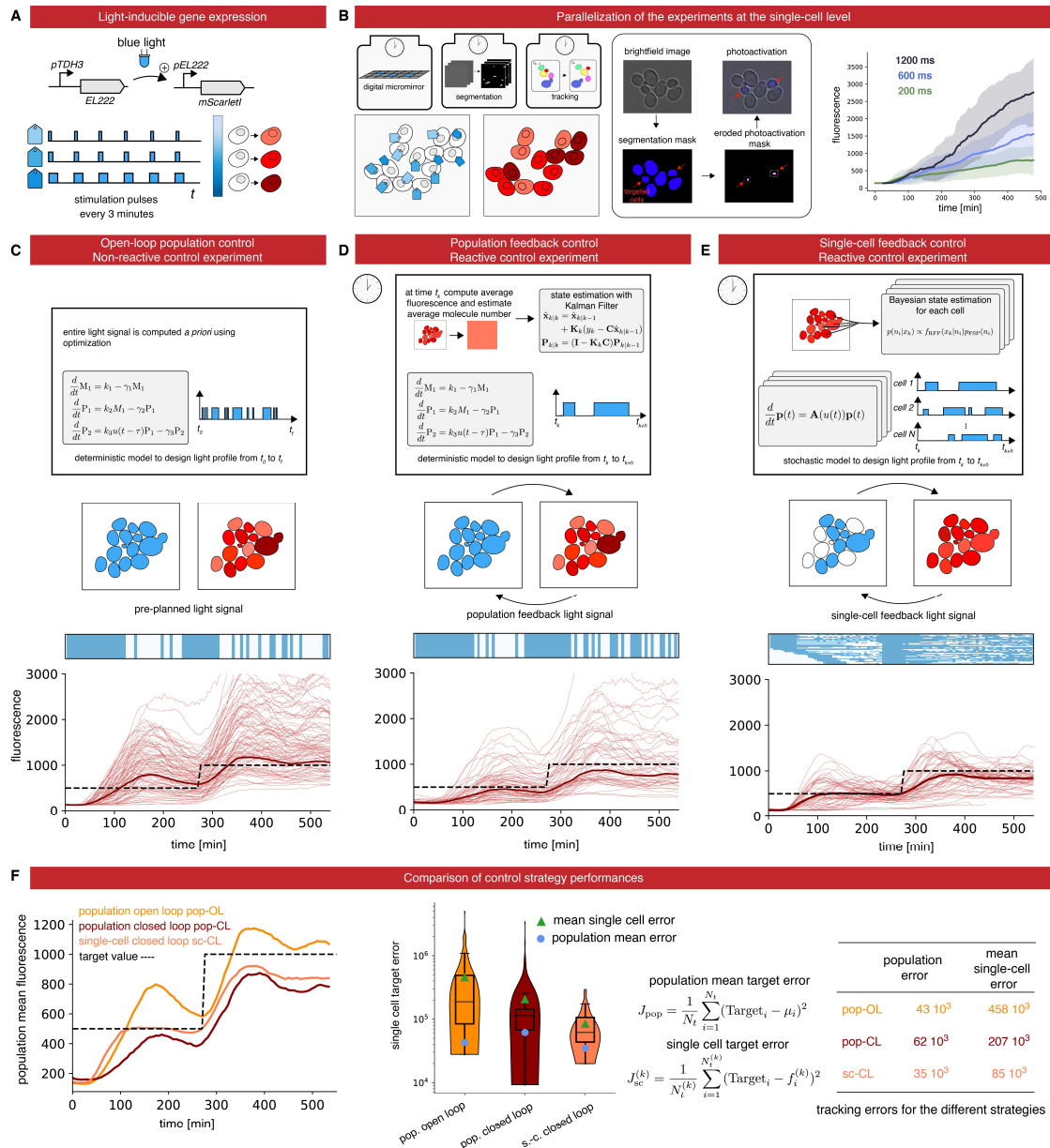
For our first application, we use the EL222 optogenetic system and the mScarlet1 fluorescent reporter to engineer light-responsive yeast cells (Fig 2A). Using real-time imaging, segmentation and cell tracking, different cells can be stimulated differently in the field of view using a digital micromirror device (DMD). Our goal is to implement different model predictive control (MPC) strategies for controlling the expression levels of a protein in a cell population. The cellular response of our engineered cells was characterized for different light stimulation profiles in the same experiment (Fig 2B). Only the most central part of the cell receives significant light stimulation. This erosion of the stimulation region helps improving the precision of single-cell light stimulations in dense cell regions because of illumination bleed-through of DMD systems (see SI Text and Fig S4). We then developed and calibrated an "average cell" (deterministic) and a "single cell" (stochastic) model of light-driven gene expression (SI Text and Fig S5, and S6).

97

In open loop control, the average cell model is used to precompute a temporal pattern of light stimulation so that cells follow a target behavior. This light pattern is then applied to all cells in the field of view (Fig 2C and Movie S2). In closed loop population-based control, the average cell model and the average of the measured fluorescence of cells are used by classical state estimators and model predictive controllers to compute in real-time the appropriate light stimulation to drive the mean fluorescence to its target (Fig 2D and Movie S3). Finally, in closed loop single-cell control, a stochastic model of gene expression and single-cell fluorescence measurements are used by advanced state estimators and controllers to compute in real-time the appropriate light stimulations to drive the fluorescence of each and every cell in the field of view to its target (Fig 2E and Movie S4). This control problem is quite challenging and needs to be solved for hundreds of cells in parallel. Advanced methods for numerical simulation and state estimation were essential (see SI Text and Fig S7).

107

108 Defining control performance as the time averaged deviation to target, we found that the single-cell
 109 control method leads to a modest reduction of error of the population averaged fluorescence but to
 110 a drastic improvement of the average error of the single-cell fluorescence (Fig 2F).



111
 112 **Figure 2. Control gene expression at the single cell level in yeast.** **A.** The red fluorescent protein mScarletI
 113 is placed under the control of the light-responsive transcription factor EL222. **B.** To efficiently characterize cell
 114 responses to light stimulations, cells in the field of view are partitioned in 3 groups, each group being stimulated
 115 with a different temporal profile. Bright-field images are segmented and cells are tracked. Then, based on their
 116 groups, cells are stimulated during the appropriate time with eroded masks. The temporal evolution of the
 117 mean mScarletI fluorescence of the cells in the three groups is shown with envelopes indicating one standard
 118 deviation. **C.** Open-loop control experiment in which a model of the response of the cell population is used to
 119 precompute a light stimulation profile that drives the cell population to the target behavior. The application of
 120 the light profile leads to significant deviations from the target of the individual cell trajectories. **D.** Closed-loop
 121 control experiment in which the same model is used jointly with real-time observations of the population state
 122 to decide which light profile to apply to all cells, using a receding horizon strategy. **E.** A stochastic model of
 123 individual cell response is used jointly with single-cell observations to decide which light profile to apply to each

124 cell. **F.** The different strategies have similar performances to drive the mean fluorescence to its target, but the
125 single-cell feedback strategy is significantly better to drive individual cells to their target profiles.

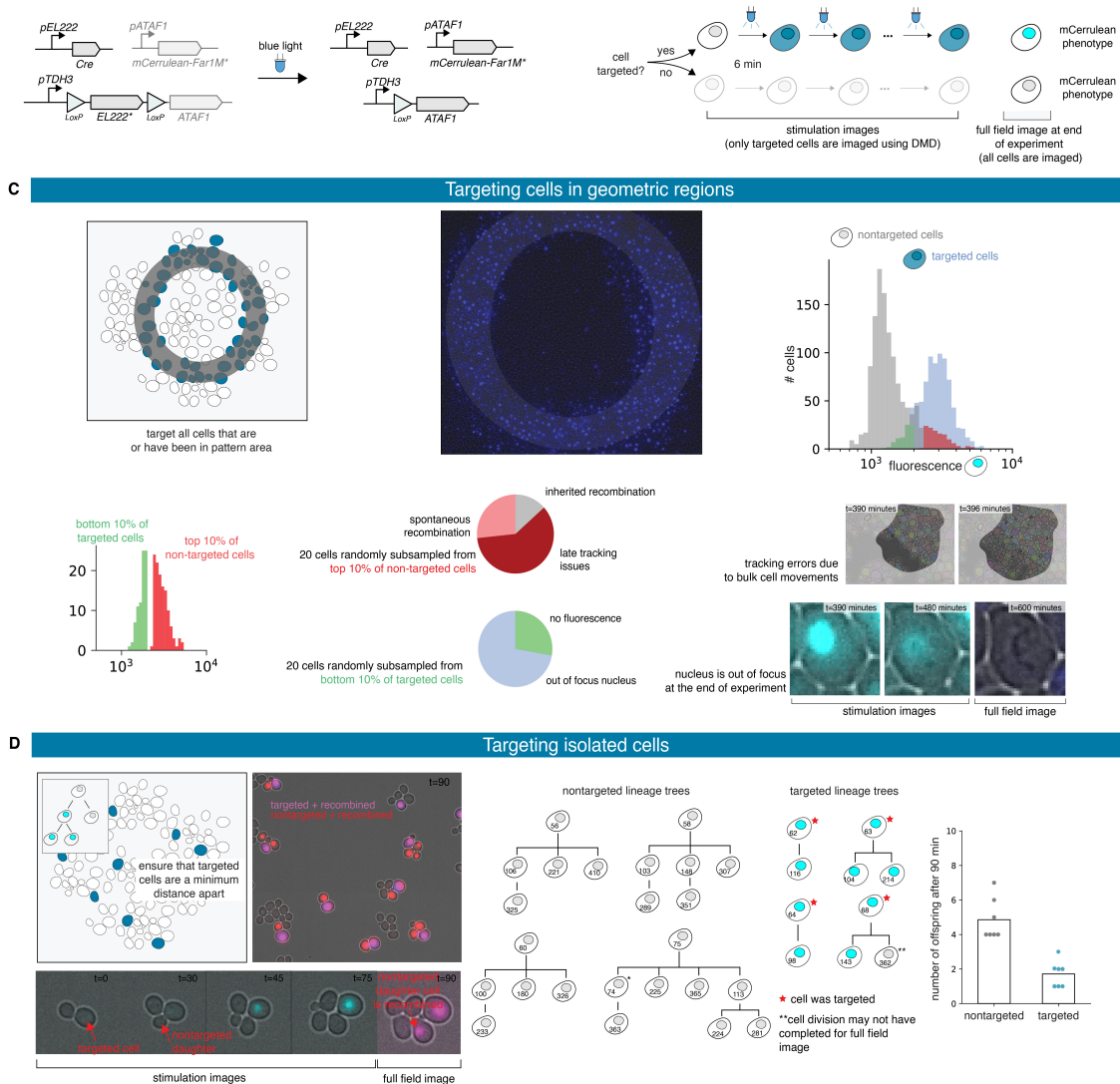
126 **Patterns of recombined yeast cells**

127 For our second application, we constructed a light-driven artificial recombination system in yeast and
128 employed different light stimulation strategies to obtain various structures of recombined cells.

129 We again used the EL222 optogenetic induction system but this time to drive the expression of the
130 Cre recombinase. The Cre recombinase induces the expression of a fluorescent reporter, mCerulean,
131 via an amplification step using the ATAF1 transcription factor (Fig 3A & 3B). This strain has been
132 designed as in Ref²².

133 Firstly, we applied a ring-like recombination signal. More specifically, every cell that was in the
134 designated zone at any moment throughout the experiment has been targeted for recombination
135 (Movie S5). As a result, we did obtain a ring-like pattern of recombined cells (Fig 3C). Experimental
136 and biological limitations can be revealed by the analysis of the tails of the distributions of the
137 recombination readout (*i.e.*, mCerulean fluorescence) within the cell populations (Fig 3C). For
138 example, we found that some cells have been erroneously targeted for recombination because of
139 tracking issues, and that only a few cells have not shown the recombined phenotype at the end of the
140 experiment despite having being effectively targeted for recombination (Fig 3C and S9).

141 Secondly, we tried to create islets of recombined cells. To this end, we dynamically searched for cells
142 that were far from any previously-targeted cell, and targeted these cells for recombination. To
143 maximize the chances that the chosen cells do recombine, we tracked each chosen cell and targeted
144 it repeatedly with light stimulations (Movie S4). Our strategy was effective in creating isolated micro-
145 colonies of recombined cells (Fig 3D). Analysis of the lineage trees of targeted cells and non-targeted
146 cells confirmed that recombined cells have a slow growth phenotype. Previous works demonstrating
147 optogenetically-driven recombination use static masks for light targeting²¹. Obtaining single-cell
148 resolution as demonstrated in Fig 3D necessitates real-time image analysis and the use of reactive
149 software.



150
 151 **Figure 3. Patterns of recombined yeast cells.** **A.** Upon light exposure, the Cre recombinase is expressed and
 152 triggers recombination, leading to the expression of ATAF1 and then of mCerulean-Far1M. Stars indicate nuclear
 153 localization of the protein. **B.** Targeted cells are stimulated for 1 second every 6 minutes until the end of the
 154 experiment. Fluorescence levels emitted by targeted cells can be recorded. At the end of the experiment, all
 155 cells are imaged and a recombined or non-recombined phenotype is attributed. **C.** A ring-like region in the field
 156 of view is selected at the beginning of the experiment and all cells entering the designated region at some time
 157 point are targeted for recombination. The distributions of the fluorescence levels of the targeted and non-
 158 targeted cells can be computed at the end of the experiment. The vast majority of cells present the expected
 159 phenotype and outliers can be further analyzed. **D.** Cells are dynamically selected such that no target cells are
 160 close to each other. Cell lineages of targeted and non-targeted cells can be manually reconstructed and statistics
 161 can be extracted.

162 Discussion

163 We presented MicroMator together with two challenging applications demonstrating how this
 164 software can help using automated microscopy platforms to their full potential. Each application goes
 165 beyond the state of the art. We showed the first demonstration of control of protein expression at the
 166 single cell level in dense field of cells. This requires to jointly solve two challenges, namely
 167 obtaining sufficiently precise single-cell stimulations with DMDs and segmenting and tracking cells

168 with sufficient accuracy over extended durations. We also provide the first demonstration of cell
169 recombination targeted at the single-cell level, enabling single-cell resolution patterns. In comparison
170 with Pycro-Manager, MicroMator uses the Micro-Manager GUI to create a main acquisition backbone
171 for the experiment and reactive events are then used to enhance or even dynamically modify this
172 initial plan. Events are created by default as separated threads and an extended logging system
173 gathers messages from all modules that might be running in parallel. This structure provides
174 robustness to real-time issues and facilitates error identification, two critical aspects for developing
175 long and complex experiments.

176 Yet, we foresee that reactivity in microscopy will primarily be used to enhance and automate
177 classical experiments. Examples of simple use cases abound: triggering autofocus only when needed,
178 dynamically adjusting the imaging condition to the signal strength, identifying novel regions of
179 interest, or following the course of experiments via easily accessible online services (e.g. warning
180 messages sent on Discord), to provide but a few examples. Thanks to its modular nature and to its use
181 of a simple but powerful event system, MicroMator capacities can be conveniently expanded to drive
182 novel hardware or perform a wide range of analyses. MicroMator is a relatively simple software
183 extension that significantly empowers laboratory equipment that is present in most quantitative
184 biology laboratory worldwide.

185 **Methods**

186 **Software and data availability**

187 MicroMator is an open-source software. It contains a core part and an extensible list of modules. The
188 MicroMator core manages the user-specified events and also the metadata and logging system. The current list
189 of modules includes a Microscope Controller module, an Image Analysis module, a Model Predictive Control
190 module, and a Discord Bot module. The Microscope Controller module is an interface with the Python wrapper
191 for MicroManager pymmcore. The Image Analysis module uses deep learning methods to segment yeast cells
192 from bright-field images. It also uses an efficient algorithm for cell tracking. This module is also available as a
193 standalone tool called SegMator. The Model Predictive Control module implements state estimation and model
194 predictive control routines for deterministic and stochastic systems, at either the population or single-cell level.
195 The Discord Bot module uses a web app running on the microscope's computer and connected to the Discord
196 communication system.

197 MicroMator, SegMator, event definitions for representative experiments (Fig 2E and S4), and data analysis code
198 for the experiments (Fig 2E, 3D, and S4), as well as a tutorial example (Supplementary Text 1), can be found
199 online: <https://gitlab.inria.fr/InBio/Public/micromator>. Raw and processed data for Fig 2C-E, 3C-D, and S4 are
200 freely available on the zenodo repository: <https://doi.org/10.5281/zenodo.4616659> (45GB).

201 **Supplementary movies**

- 202 • **Movie S1:** `real-time_segmentation_and_tracking_with_SegMator.mov`. Time-lapse movie showing the real-
203 time segmentation and tracking quality obtained with SegMator. Left: bright-field image. Right: bright-field
204 image overlaid with segmentation mask in cyan.
- 205 • **Movie S2:** `optogenetic_control_of_gene_expression-Open_loop.mov`. Time-lapse movie showing the
206 response of the cells (mScarlet1 fluorescence) in an open-loop control experiment. Corresponds to Fig. 2C.
- 207 • **Movie S3:** `optogenetic_control_of_gene_expression-Population_closed_loop.mov`. Time-lapse movie
208 showing the response of the cells (mScarlet1 fluorescence) in a population closed-loop control experiment.
209 Corresponds to Fig. 2D.
- 210 • **Movie S4:** `optogenetic_control_of_gene_expression-Single_cell_closed_loop.mov`. Time-lapse movie
211 showing the response of the cells (mScarlet1 fluorescence) in a single-cell closed-loop control experiment.
212 Corresponds to Fig. 2E.
- 213 • **Movie S5:** `single_cell_recombination-Islets.mov`. Time-lapse movie showing the light signal sent to cells in
214 order to create small islets of recombined cells. Corresponds to Fig 3C.

- 215 • **Movie S6:** single_cell_recombination-Ring.mov. (Left) Time-lapse movie showing the light signal sent to cells
216 in order to recombine all cells that have been at one moment in a ring-like pattern. (Right) Image showing
217 the recombined state of the cells at the end of the experiment. Corresponds to Fig 3D.

218 **Genetic constructions and yeast strains**

219 All plasmids and strains were constructed using the *Yeast Tool Kit*, a modular cloning framework for yeast
220 synthetic biology²³, the common laboratory strain BY4741 (Euroscarf), and the EL222 optogenetic system²⁴. The
221 light responsive strain (IB44) harbors a constitutively expressed EL222 light-responsive transcription factor (NLS-
222 VP16AD-EL222) and an EL222-responsive promoter (5xBS-CYC180pr) driving the expression of the mScarlet1
223 protein. The IB44 strain genotype is MATa his3Δ1 leu2Δ0::5xBS-CYC180pr-mScarlet1-Leu2 met15Δ0 ura3Δ::NLS-
224 VP16AD-EL222-URA3. The recombining strain (IB237) harbors a constitutively expressed EL222 light-responsive
225 transcription factor (NLS-VP16AD-EL222) floxed between two LoxP sites that upon recombination expresses the
226 ATAF1 transcription factor. This factor expresses (pATAF1_4x) in turn the mCerulean fluorescent protein fused
227 to a constitutively active Far1 protein (FAR1M_mCerulean). Lastly, the strain also harbors the Cre recombinase
228 placed under the control of an EL222-responsive promoter (5BS-Gal1pr). The IB237 strain genotype is MATa
229 his3Δ1::pATAF1_4x-FAR1M_mCerulean-tDIT1-HIS3 leu2Δ::5BS-Gal1pr-CRE-tENO1-LEU2 met15Δ0 ura3Δ::
230 pTDH3-LoxP-NLS-VP16AD-EL222-tENO1-LoxP-ATAF1-tTDH1-URA3. Lastly, we also used the IB84 strain as a
231 constitutive 3-color strain to characterize DMD precision. The genotype of this strain is MATa his3Δ1
232 leu2Δ0::pTDH3-mCerulean-tTDH1-pTDH3-NeonGreen-tTDH1-pTDH3-mScarlet-tTDH1-LEU2 met15Δ0 ura3Δ::
233 NLS-VP16AD-EL222-URA3.

234 **Culture preparation**

235 Cells were grown at 30°C in synthetic medium (SD) consisting of 2% glucose, low fluorescence yeast nitrogen
236 base (Formedium CYN6510), and complete supplement mixture of amino acids and nucleotides (Formedium
237 DCS0019). For each experiment, cells were grown overnight in SC media at 30°C, then diluted 50 times and
238 grown for 4 to 5 hours before being loaded in microfluidic plates.

239 **Microscopy setup, microfluidics and imaging**

240 Images were taken under a Leica DMI8 inverted microscope (Leica Microsystems) with a ×63 oil-immersion
241 objective (HC PL APO), an LTM200 V3 scanning stage, and an sCMOS camera Zyla 4.2 (ANDOR). Bright-field
242 images were acquired using a 12V LED light source from Leica Microsystems. Fluorescence images were acquired
243 using a pE-4000 light source from COOLLED and the following filter cubes: EX:436/20nm DM:455nm
244 EM:480/40nm (CFP), EX:500/20nm DM:515nm EM:535/30nm (YFP), and EX:546/10nm DM:560nm
245 EM:585/40nm (RHOD) from Leica Microsystems. Light stimulation was performed using the pE-4000 light source
246 and the CFP filter. Spatially-resolved illuminations were obtained thanks to a digital mirror device (DMD)
247 reflecting the light of a pE-4000 light source. We used a MOSAIC3 DMD from ANDOR. The device is used both
248 for targeted fluorescence imaging and for optogenetic stimulations. A CellASIC ONIX2 system (Merck) was used
249 together with the Y04C CellASIC microfluidic plates to grow yeast cells in monolayers. Media flow was
250 maintained by a 7.5 kPa pressure gradient. The media was the same as for pre-culture. The temperature was
251 maintained at 30 °C by an opaque environmental box and a temperature controller 2000-2, both from PECON.
252 The microscope was operated using MicroMator.

253 **Model predictive control of gene expression**

254 To compare single-cell and population control strategies, we developed stochastic and deterministic models of
255 gene expression. Both have been calibrated with respect to the dataset presented in Fig 2B and Fig S5. For
256 population control, we used the deterministic model, assumed Gaussian measurement noise and used a Kalman
257 filter for state estimation. Each model assumes a deterministic delay between the time the light signal is applied
258 and the time protein production is effective. For MPC, fluorescence measurements were taken every 6 minutes
259 and we considered receding time horizons of 24 minutes. The controller explores the set of light stimulation
260 profiles in which a 1000ms light stimulation is either applied or not for each measurement time interval, and
261 selects the profile minimizing mean square deviations. For tracking purposes, brightfield measurements were
262 taken every 3 minutes. For single-cell control, we used the stochastic model and simulated the cell behavior
263 using a finite state projection approximation. For each and every cell, state estimation is performed using a

264 Bayesian approach which conditions the probability distribution for each cell on the most recent measurement,
265 and light stimulation profiles are selected using the approach outlined above and the expected absolute
266 deviation as selection criterion. More information is provided in SI Text. Box plots of Figure 2F indicate the lower
267 quartile, the median, and the upper quartile of the data, with the whiskers corresponding to 1.5 interquartile
268 ranges.

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324 **Author contributions**

325 S.F. developed the MicroMator software and performed experiments. Z.F. wrote the image analysis and real-
326 time control modules, and analyzed the data. A.F. performed experiments and helped develop the MicroMator
327 software and analyze the data. C.A. developed strains and helped perform experiments. S.S.-C. developed
328 strains. S.G. helped with software development. F.B. helped with software development and platform
329 integration. J.R. helped with controller development. Z.F., F.B., J.R., and G.B. supervised the project. Z.F. and
330 G.B. wrote the manuscript with input from all authors.

331 **Declaration of interests**

332 The authors declare no competing financial interests.